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NUTRITION REVIEWS

October 1957

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NUTRITION REVIEWS

VOL. 15 OCTOBER 1957 No. 10

CHANGING PATTERNS IN NUTRITION EDUCATION

With the accumulation of information in nutrition, the scope and functions of nutrition education have broadened in spectacular fashion. Dr. L. A. Maynard (*Nutrition Reviews* **9**, *354 (1951)*) said: "An over-all program of nutrition education must do more than teach the individual good eating habits. It should also develop a public consciousness of the importance of good nutrition and of sound food policies for the promotion of our national vigor and stability. Ill-fed people do not make good citizens. Employers need a greater appreciation of how good nutrition increases work efficiency and cuts down absenteeism. Taxpayers need to realize that good nutrition decreases public expenditures for medical care and for the support of those unable to earn their own living."

To meet these new objectives nutrition education is in the process of changing from a passive, academically interesting subject, to a dynamic area of learning directed toward the welfare of individuals and of nations, and of future, as well as present, generations. Because the body of knowledge in nutrition lends itself naturally to classification, a conventional, subject-matter-centered approach to teaching nutrition has evolved. This approach, admirably adapted to higher education, has been very effective in stimulating scholarly attainments, but less effective in influencing people's use of food. The present paper is concerned with nutrition education designed to serve the needs of the masses of people.

Gradually the focus in nutrition education is changing from a subject-centered to a person-centered, or even a society-centered, area of learning. In its application there is a growing recognition of the need to use the knowledge and skills of the educator as well as those of the nutrition scientist. With the increasing scope and technicality of the subject of nutrition, the two are seldom one person. The nutrition educator is confronted with the difficult task of mastering the subject matter in nutrition and developing skill in the using of tools of education. The effective application of nutrition knowledge demands basic understandings from the fields of anthropology, psychology and sociology. These must be attained in addition to a comprehension of nutrition which will enable the educator to keep up-to-date and meet uncharted situations and problems.

At The Nutrition Education Conference, April, 1957 (Jointly sponsored by the U. S. Department of Agriculture Nutrition Committee and the Interagency Committee on Nutrition Education and the School Lunch, Washington, D. C.), Dr. Robert Fleming, (Chairman, Department of Early Childhood and Elementary Education, School of Education, New York University, N. Y.) urged nutrition educators to make use of the basic principles of learning, which for practical purposes he grouped in five "clusters": (1) Placing emphasis on the individual, his own background, differences, and goals; (2) considering the learner's own perception of the task to be accomplished and his "readiness" to learn the lesson to be taught; (3) recognizing the importance of human relations in the learning situation, that is realizing that people tend to learn in an atmosphere that is friendly, relaxed, and secure; (4) involving the learner; and (5) making wise use of the many materials and resources now available. These clusters, he said, are suggestive of forces in "the teaching-learning process", that have important implications for nutrition teaching.

Modern educational philosophy advocates relating the teaching to the goals and purposes of those being taught. People are more

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likely to adopt nutrition principles or to change food habits when they believe that the action will help them achieve their own important goals. Nutrition teaching should enable people to recognize the advantages of changing their food habits. In following this mode of action, two major problems arise: (1) lack of knowledge about the values and goals of people and (2) difficulty in relating nutrition to anything so complex as a goal or objective of a human being.

Nutrition education has been geared to the idea that people, regardless of age, are concerned about health or being strong, about growing or having healthy children, or about delaying the onset of old age. At some of the most critical periods in nutrition education, these worthy goals have little appeal. Rather, concern for personal appearance, popularity, or athletic prowess may be the dominant interest. At any rate, efforts to ascertain the goals of individuals and to take advantage of "readiness to learn" and "teachable moments" will be rewarding to the nutrition educator.

In organizing subject matter in such a way that it conforms to the aims and goals of individuals, information must be carefully sifted and the contributions of nutrition evaluated. The research point of view is vital to this approach. The way must be paved for the learner to accept new information with which to revise the old. In this aspect of nutrition education it is important to have some appreciation of the potentialities and limitations of nutrition in the entire health picture, and of the interaction among the forces which lead to the attainment of most goals. The contribution of nutrition to the well-being of people has emphasized chiefly the physical. Future work in nutrition education requires an answer to the question of how the amount and kind of food eaten, and the distribution of foods among meals and snacks, affects the mental as well as the physical characteristics.

A first step toward engaging the interest of a group of people is to convince them that a problem exists in their midst. The nutrition survey has therefore become one of the tools in nutrition education. In 1947, Dr. L. B. Pett (*J. Am. Dietet. Assn.* **23,** *13 (1947)*) challenged nutrition education as being too general. He stated that it has consisted of a "shotgun" or "blunderbuss" approach; that we have dealt too much in national averages and too little in individual situations. He recommended the approach based on a definite knowledge of conditions in the home and community, and carried into effect by individuals in the community.

The survey may be designed to ascertain facts about the total diet, about certain meals or snacks, about habits or attitudes, or about the nutritional status of the population involved. Unless a nutritional survey is directed by competent persons the results may be misleading. The interpretation of survey information requires skill and understanding.

Because foods are better understood by people than are nutrients, food groups have been widely used in teaching nutrition. This approach was an outgrowth of the concept of "protective foods" first voiced 30 to 40 years ago. The usefulness of this device lies in its emphasis on the exchangeability of foods and the general nutritional properties of food groups. Its limitation lies in the tendency to belittle the importance of acquiring needed information about the calorie and nutritive value of foods, and the variety of sources from which certain nutrients may be obtained. Then too, individuals failing to understand the many ways to attain a good diet, have been bewildered by the appearance of many different food guides.

The results of dietary surveys are often interpreted in terms of apparently neglected food groups, and the nutrition education program is directed toward changing the food habits as indicated by the survey. This may be an effective procedure, but it should not replace teaching the essentials of an adequate diet. Once the facts about diets

or nutritional status are well defined by survey methods, careful study of the reasons for existing conditions should be made. Emphasis on neglected food groups without concern for the reasons why they have been neglected will not lead to permanent change.

At the Nutrition Education Conference previously mentioned, Dr. John Cassel (*Am. J. Pub. Health* **47** *732 (1957)*), expressed concern that nutrition educators consider the problem of changing food habits in relation to the "social and cultural components of the social environment". The social scientist, he pointed out, can help the nutritionist in understanding the many ramifications of food habits and customs, and in predicting the long-range aspects of change. He used a case study taken from the health program initiated with the Zulu tribesmen of South Africa in 1940 to illustrate the possibility of changing deeply rooted food habits and of eventually attaining desired goals without catastrophic effects on the culture patterns of the group.

Briefly, he adduced two basic principles which in essence were: (1) health workers should have an intimate detailed knowledge of the peoples' beliefs, attitudes, knowledge and behavior before attempting to introduce any innovations into an area; (2) they should evaluate the psychologic and social functions of the practices, beliefs and attitudes of the people and the patterns or systems into which the customs or beliefs fit.

Nutritionists, according to Cassel, should be aware that even within our so-called overall "American culture" numerous distinct subcultures exist, which differ in their attitudes, beliefs, knowledge, and practices in regard to food.

Cassel also expressed concern about "culture bound" health workers, who are convinced that their own set of beliefs, attitudes and practices are the only correct ones and about those who think that a population with no knowledge of our concepts of nutrition has no ideas whatever concerning it. The recognition in nutrition education of these problems and principles advanced by the social sciences will do much to bring it down-to-earth and make it live.

A useful analysis of the process by which people accept new ideas has resulted from a 20-year research project conducted with farm people in various parts of the United States (*How Farm People Accept New Ideas.* North Central Regional Publication No. 1 of the Ag. Extension Service. Special Report No. 15, Ag. Ext. Service, Iowa State College, Ames, Iowa, November, 1955.) The findings indicate that the mental process consists of at least five stages and that individuals can distinguish one stage from another and designate points in time when they went through each stage. In number one, the awareness stage, the individual merely knows about the existence of the idea. At the second, the interest stage, the individual wants more information on how he can be helped, how the idea works, and what its potentialities are. The third is the evaluation stage, in which he asks himself if he can do it, and if he can, will he be better off than before. The fourth is the trial stage, characterized by small scale experimental use and by need for specific information. The final stage is the adoption stage. In it is the large scale continued use of the idea and satisfaction with it. As the educator develops the ability to identify these stages and provide appropriately for them, he may expect to meet with greater success in his program. Further information concerning the factors that influence the rate at which people pass through these stages may offer important clues in nutrition education, although the research as yet has not included a study of the adoption of new practices in nutrition.

Nutrition education has been dispensed largely through the lecture method. Well-illustrated talks will probably always have a place, but most learners should be involved in other ways than merely listening to talks. Involvement occurs when people evaluate their own dietary habits, their own nutri-

tional status, or when they may find out for themselves the effects of dietary changes on growth or well being. Group discussions, sometimes followed by decision, and group efforts directed toward a common problem are currently employed as means of involving people in learning nutrition.

The fast pace of education through television, radio and various advertising media is making an impact on nutrition education. Many materials are available. The up-to-date nutrition educator keeps abreast of these and makes effective use of them, and, in addition, develops dramatic visual aids appropriate to the immediate presentation. An element of the dramatic is important in nutrition as in all kinds of education. The early emphasis on nutritional deficiency diseases was in this respect more effective than the later, positive approach of "abundant health." The judicious use of illustrations of the effects of drastic dietary deficiencies should perhaps not be abandoned.

Dr. H. C. Sherman has said (*Chemistry of Food and Nutrition, 8th ed., Macmillan, 1952*) that nutrition is a subject that appeals both to the *spirit of wonder* and to the *spirit of service*. Nutrition education has been more effective in revealing the wonders of nutrition than in bringing about its everyday application to the betterment of mankind. With the growing appreciation of the importance of nutrition education, and the development of better understanding of the basic problems in teaching nutrition, progress may be expected.

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CHEWING YOUR FOOD

The past decade has seen a revival of interest in mastication as it relates to a variety of physiological conditions. One of these fields of interest has been the possible relation between dental caries and mastication. As a result of the dental surveys made in Europe during and following World War II, a number of investigators attributed the reduction in dental caries in the occupied countries to the increased chewing necessitated by the coarse foods eaten (*Nutrition Reviews* **8**, *344 (1950)*; **10**, *333 (1952)*). The validity of these claims has been challenged by other investigators who found no relation between the hardness of the diet and the incidence of dental caries in cotton rats (*Ibid.* **13**, *177 (1955)*).

Another area of interest has been the reported influence of the teeth upon an individual's nutritional status. From a clinical study of patients, H. I. Greene, S. Dreizen and T. D. Spies (*Ibid.* **8**, *149 (1950)*) concluded that "an intimate relation exists between impaired masticatory function (masticatory insufficiency) and general nutritive failure." As the original reviewer pointed out, the data presented by the above workers failed to justify their conclusion.

From time immemorial there has been an interest in the effect of chewing upon the relative digestibility of foods. The enthusiasm for chewing food probably reached its peak about one-half century ago when Horace Fletcher had a large following who accepted his tenet that food must be chewed at least 32 times (E. V. McCollum, E. Orent-Keiles and H. G. Day, *Newer Knowledge of Nutrition, MacMillan Co., New York, p. 568 (1937)*). Fletcherism had an earlier apostle in the British Prime Minister Gladstone, who attributed his success in life largely to the fact that he had made it a rule to give every tooth a chance; to do that, he had applied 32 bites to every mouthful. As McCollum and his associates (*Loc. cit.*) point out, astute statesman that he was, Gladstone

failed to observe that "with a full dental complement," 16 chews would have served his purpose."

In England J. H. Farrell (*Brit. Dent. J.* **100,** *149 (1956)*) was impressed by the fact that the feces of persons with good dentition frequently contained large and obviously unchewed portions of food. In order to study the effect of chewing upon the degree of digestion, he cut food into cubes approximately 1 cm. on an edge. Half of each food item was chewed by the individual without swallowing. The chewed food was put into a mesh bag (1 mm. mesh), while the unchewed food was put into another mesh bag. Small ball bearings were put into one of the bags in order to distinguish the chewed from the unchewed food. These bags were then tied together and swallowed by the 10 subjects, all of whom were adults in good health who had never suffered from any gastrointestinal disturbance which was likely to have an effect on their suitability as a subject for the experiment.

The bags were swallowed by the subjects at the meal following the daily bowel movement. The feces passed during the next 24 to 36 hours were saved for recovery of the cotton bags. Farrell (*Loc. cit.*) claimed that the bag into which the chewed food had been put was always empty, suggesting that as the food was digested, the solubilized material left the bag, which in turn collapsed, thus preventing the entrance of any particles.

Some foods, such as roasted and fried meats, showed a much higher per cent of residue for the unchewed food compared to the chewed food. The differences in the percentage residue for the meats ranged from 24 to 63. In the same category were fried and boiled potatoes, boiled peas, and boiled carrots. Other foods, such as roast chicken, stewed lamb, fried and boiled fish, hard-boiled eggs, boiled rice, white bread, and cheese, showed no difference between the chewed and unchewed samples.

Photomicrographs accompanying the paper showed distinct cellular tissue for a variety of the meats tested. These unchewed samples went through the gastrointestinal tract while maintaining their cellular integrity in the residue recovered from the feces.

Although the author claims that the mesh bags had no adverse influence on the digestive processes, one might question this on the following grounds: The subjects had difficulty in swallowing the bags. What effect the psychological reactions resulting from this had on the gastrointestinal tract is difficult to tell. In the second place, all of the subjects were given a laxative eight hours after swallowing the bags. If this were not done, it was found that the bags remained for a considerable time in the large intestine. Under such circumstances, the bags, when recovered from the feces, showed no residue, even for the unchewed foods which left the highest residue with the laxative. It is likely that only minimal absorption occurs from the large intestine, but such a possibility cannot be ruled out (O. Mickelsen, *Vitamins and Hormones* **14,** *1 (1956)*).

In the same study individuals showing wide variations in their masticatory efficiency were studied by the above method. The masticatory efficiency was measured by the method of R. S. Manly and L. C. Braley (*J. Dent. Res.* **29,** *448 (1950)*). According to this method, the efficiency is expressed as the percentage of chewed, unswallowed salted peanuts that pass through a 10 mesh screen. The results with three different subjects indicated that even with very poor masticatory efficiency (only two posterior teeth occluded) the amount of residue remaining in the bag recovered from the feces was no greater than that from individuals with good masticatory ability. In all of the latter experiments fried pork was used, since it previously had been shown to leave a large residue. All three subjects chewed each specimen of pork 20 times.

In an extension of the above work, J. H. Farrell (*Brit. J. Nutrition* **10,** *111 (1956)*) found that when lean beef was prepared for

frying by pounding with a spiked hammer and then treated with a proteolytic enzyme from papaya juice, the unchewed meat was digested to a much greater extent than was the case when the meat was not treated prior to frying. Meat that, prior to frying, had either been soaked in dilute acetic acid, beaten with a rolling pin, or treated with a proteolytic enzyme alone showed as much residue in the bags recovered from the feces as the untreated meat.

It would seem that the difficulties associated with the use of the mesh bags might be avoided if the subjects were given a bland diet containing fairly large amounts of the test food. The stool samples could be analyzed for protein and fat while on the bland diet and also while receiving this diet supplemented with fairly large amounts of the test food.

These studies are interesting in that they provide at least an experimental approach to the problem raised by the relation of dental health to digestive processes. The results indicate clearly that even in an individual with extreme limitations of masticatory efficiency, the undigested residue remaining is negligible.

ANEMIAS OF THE TROPICS

H. Foy and A. Kondi (*J. Trop. Med.* **60,** *105 (1957)*) summarized their data on more than 7,000 hemoglobin determinations upon adults from rural working populations in Africa and India. In India the subjects were laborers on tea gardens, and peasant farmers from outlying villages; in Africa peasant farmers growing rice, maize, cotton, and similar crops, or workers on sisal and sugar plantations were the subjects. Infants, invalids, or very aged persons were excluded from the sample. The places included tropics and subtropics varying in altitude from sea level to 8,000 feet with annual rainfalls that varied from a few to several hundred inches.

In the hot damp river valleys of India (Assam, with an altitude of 300 to 600 ft.) the mean hemoglobin level for all groups was 10.9 g. per 100 ml. with 16.5 per cent of the population below 8 g. per 100 ml. The lowest levels were in pregnant women with mean values of 9.4 g., and 29 per cent below 8 g. per 100 ml. Lactating women were approximately the same as non-lactating women, the latter having a mean value of 10.9 g. with 15 per cent below 8 g. per 100 ml. Men in the same area had a mean value of 12.7, with 6 per cent below 8 g. per 100 ml. In Portuguese East Africa, with a similar altitude but somewhat hotter and more extended dry season, over 3,000 persons were examined. The situation was somewhat worse, with an overall mean level of 10.1 g. per 100 ml. and 32 per cent below 8 g. Pregnant and lactating women (not separated) averaged 9.0 g. and 41 per cent had values less than 8 g. per 100 ml.

At higher altitudes (Darjeeling, altitude 2,500 to 8,000 ft. and Nilgiri Hills, altitude 3,000 to 6,000 ft) the values were somewhat higher. The mean hemoglobin levels were 12.2 and 11.7, respectively, with 13 and 18 per cent below 8 g. per 100 ml. Pregnant women again had mean values from 1 to 2 g. below the mean of the total group, with approximately 20 per cent below 8 g. Men averaged approximately 14 g. per 100 ml. The values in Ceylon at altitudes of 2,500 to 7,000 ft. were about 1 g. higher (pregnant women, 11.8, lactating and non-lactating women, 12.6, and men, 15 g. per 100 ml.). It is apparent that severe anemias are widespread, the range of values encountered in all areas being from 4 to 17 g. per 100 ml.

The types of anemias are to be described elsewhere. However, the authors state that 85 per cent had normal to low mean corpuscular volumes, low mean corpuscular hemoglobins, and mean corpuscular hemoglobin concentrations with erythronormo-

blastic marrows. They are said to be iron deficient and to respond to 15 to 35 grains of oral ferrous sulfate daily for 2 to 4 months. The remaining 15 per cent had normal, high or low M.V.C., M.C.H's., and M.C.H.C's., and megaloblastic marrows and responded to folacin for vitamin B_{12}.

Foy and Kondi discuss the possible etiology extensively. They point out that the somewhat higher hemoglobin levels at higher altitudes are a common experience, but do not necessarily indicate any diminution in anemia. The possible important factors to be considered in the genesis of such iron deficiency are (a) poor absorption due to the type of diet, (b) excessive dermal losses and other losses due to climate, (c) pregnancy and lactation, (d) blood loss due to intestinal parasitism, and (e) quantitative and qualitative changes in the protein in the diet.

The authors quote V. E. Henderson and G. H. W. Lucas (*Am. J. Digest. Dis.* **11,** *244 (1944)*) and C. V. Moore (*Am. J. Clin. Nutrition* **3,** *3 (1955)*) to the effect that in the European and American diet from 12 to 20 mg. of iron is supplied daily and an absorption of only 3 to 10 per cent of this is regarded as sufficient for both men and women. They then state that approximately 27 mg. of iron is needed daily for hemoglobin synthesis, of which about 20 mg. are supplied by hemoglobin breakdown and the remaining 7 mg. must be supplied either by iron stores or by diet. The basis for the latter estimate is not given and will undoubtedly be disputed by various workers in the field. According to available data the iron content of Indian diets is generally between 12 and 40 mg., with a considerable number above 60 mg. daily. High iron intakes are also common in Africa. The authors describe the known effects of phytic acid in inhibiting iron absorption and suggest that this may be of importance in the higher cereal diets consumed in these areas. The bacterial flora, the bulk of the diet, the calcium to phosphorus ratio, gastric acidity, etc., are also suggested as worthy of consideration.

With regard to iron losses the authors emphasize the losses through the skin. In their own work (Foy and Kondi, *Lancet* **II,** *95 (1956)*) they have found that sweat may contain from 0.3 to 6.0 mg. of iron. Most of this iron was found in the desquamated epithelial cells, since a cell-free supernatant of sweat contained only 100 to 200 micrograms per liter. Losses of this magnitude agree with the results of H. H. Mitchell and T. S. Hamilton (*J. Biol. Chem.* **178,** *345 (1949)*) and W. S. Adams *et al.* (*Proc. Soc. Exp. Biol. Med.* **74,** *46 (1950)*). However, R. Dubach *et al.* (*Blood* **3,** *526 (1948)*) and F. A. Johnson *et al.* (*J. Nutrition* **43,** *285 (1950)*) report losses by this route to be much less, of the order of 0.5 to 2 mg. daily. The authors believe such low values to be due to the use of filtered or cell-poor sweat.

Since the volume of sweat in the tropics may vary from 2 to 11 liters, and the authors state that the cell content may vary from 0.8 to 3.5 per cent of the sweat, it is clearly important to obtain a satisfactory resolution to this problem. As the authors point out, once iron is deposited in the epithelial cells it is probably no longer available to the body. If this is true, then the rate of regeneration of the stratum granulosum is the important factor rather than the sweating rate, unless the regeneration rate is a function of the temperature or amount of sweat produced. The authors also state that the distribution and number of apocrine and eccrine glands differ in Europeans, Indians and Africans. The volume and composition of sweat may also vary.

Menstrual and pregnancy iron losses are undoubtedly important, as evidenced by the more severe anemias in women and especially in pregnant women. According to the literature the menstrual iron losses are extremely variable, ranging from 3 to 450 mg. Most authors find the largest losses to be of the order of 80 to 100 mg. and average losses are probably about 20 mg. On the other hand,

L. S. P. Davidson *et al.* (*Brit. Med. J.* **1,** *685* (*1933*)) found a greater proportion of anemia among women with a history of menorrhagia. The authors have no evidence that menorrhagia is commoner in Indian and African women than among Europeans, nor is it known whether excessive functional uterine bleeding is more common among these groups.

During pregnancy the authors suggest that from 30 to 400 mg. of iron is saved by the cessation of menstruation and the fetus requirement is from 300 to 500 mg. There is thus an apparent increased need of some 200 to 300 mg. of iron. To this may be added another 200 to 300 mg. to account for losses in the placenta and the blood lost during parturition. There is apparently no reason to believe that such losses are greater in the tropics. However, the authors believe that early marriages resulting in pregnancies while growth and expansion of blood volume are still occurring is an important factor, especially in India.

It is of interest that the authors state that in many cases pregnant women with hemoglobin values of 4 g. per 100 ml. went into normal labor and produced infants whose hemoglobin levels were not significantly different from infants whose mothers had normal hemoglobin values. Although they did not specifically study such infants further, it is their impression that, if the infants were not premature, their progress, morbidity and mortality would not be worse than those from normal mothers.

The authors believe, mainly from a literature review but also from their own data in India, that hookworm is not a major factor in the causation of these iron deficiency anemias. However, the relationship of parasitic infestations to diet, sex, iron losses, etc., still requires much more critical work. Malaria also is not a major problem in the areas in which these data were collected.

Finally, the role of protein deficiency is considered. While this may play a role, the evidence indicates that it is only a minor one. Two types of non-pernicious megaloblastic anemias in Africans have been described previously (Foy, Kondi and P. E. C. Manson-Bahr, *Lancet* **II,** *693* (1955)). One is said either to respond to penicillin or to vitamin B_{12} orally, and is apparently a true vitamin B_{12} deficiency. The other responds to folacin.

Foy and Kondi point out that very little is known about the economic importance of anemia. While it might be expected that anemia would lower the working efficiency, "it is common to see people in India working with hemoglobins as low as 4 g. per 100 ml., many of them pregnant or lactating. Is the work of such grossly anemic people less efficient than those with normal hemoglobin levels?" In Western Europe and America individuals with a 10 per cent reduction in hemoglobin are regarded as unwell. "In the tropics pregnant women with less than 4 g. per 100 ml. carry out their normal daily routine without complaint and if questioned will nearly always say they feel all right."

Since 85 per cent of the anemias respond to iron administration, the authors suggest that some thought should be given to the fortification of the diet with iron.

PHENYLKETONURIA

Phenylketonuria has become the common name for that inborn error of metabolism previously known as phenylpyruvic oligophrenia because the presence in the urine of the keto acid derived from phenylalanine, phenylpyruvic acid, is the major initial diagnostic finding in these patients. A description of this disease and the first attempts to control its manifestations by dietary means have previously been reported (*Nutrition Reviews* **14,** *42* (*1956*)).

In a recent review by S. W. Wright and G. Tarjan (*Am. J. Dis. Child.* **93,** *405* (*1957*)) the statement is made that phenyl-

ketonuria may well become a working model for the study of diseases in which psychological abnormalities and multiple biochemical defects are the result of a genetically determined enzyme deficiency. The susceptibility of certain inborn errors of metabolism to dietary control is also represented by galactosemia (*Nutrition Reviews* **15,** *159 (1957)*). In their review Wright and Tarjan point out that, although the I.Q. in the majority of patients is less than 20, serious mental retardation is not necessarily present in this disease, several known individuals with intelligence within the normal range having been reported. The characteristic dilution of the hair, eye, and skin color mentioned by many authors was absent in several of their cases which were personally observed. The incidence of this disease has been calculated as 0.64 per cent among institutionalized mentally defective patients by G. A. Jervis (*A. Res. Nerv. Ment. Dis., Proceedings* **33,** *259 (1954)*). The frequency of the disease has been estimated as occurring in two to six persons of 100,000, placing the frequency of the heterozygous carrier at one in 80 of the general population. There seems to be consistent agreement that inheritance of this disease progresses as a simple recessive. Wright and Tarjan present a very useful tabulation of the chances of affected children from matings of relatives of patients with this disease. Since recognition of these patients is difficult on clinical grounds, testing of the urine with ferric chloride on more than one occasion is essential for adequate diagnosis. This has become of more than academic interest with the availability of treatment of this condition.

The effect of a phenylalanine-restricted diet in phenylketonuria in six cases has been reported by F. A. Horner *et al.* (*Am. J. Dis. Child.* **93,** *615 (1957)*). The phenylalanine-restricted diet used by these authors consisted of a phenylalanine-free protein hydrolysate fed at a level of 4.2 g. per kg. per day, fat in the form of a vegetable oil (3.0 g. per kg. per day), and a suitable carbohydrate at a level of 10 g. per kg. per day. On such a diet the serum phenylalanine level has been shown to return from abnormally high to normal levels. After the initial three-week period phenylalanine in the form of natural protein is added to the diet in sufficient quantity to maintain the serum phenylalanine level between 3 and 5 mg. per cent (usually 3 to 8 g. of protein per day). In their first patient, aged 4¼ years, psychological evaluation showed extreme hyperactivity, no language, no social control, and a development quotient of 35. After the first 14 months of treatment his development quotient had increased to 69, with an increment of mental age of 26 months in this period. After 22 months of treatment he played quietly, talked in meaningful sentences and controlled his aggressive impulses most of the time. In a second 4-year-old patient similar results were reported, with a gain of 35 months in developmental age during 20 months of treatment. A sibling of the second patient was found to have phenylketonuria at the age of 7 weeks, at which time his serum phenylalanine level was 55 mg. per cent. Treatment was instituted and at the age of 20 months, his development quotient was 91 and his developmental pattern compared favorably with that of his older, unaffected brother. Less remarkable responses to the phenylalanine free diet were seen in two other patients, aged 17 months and 11 months.

Their sixth subject was a sibling of one of the patients with a relatively poor response to treatment. Detailed studies of the serum phenylalanine levels in this infant have been reported by M. D. Armstrong and E. L. Binkley, Jr. (*Proc. Soc. Exp. Biol. Med.* **93,** *418 (1956)*). Serum phenylalanine determinations in cord blood and in venous blood at intervals of three or four days were obtained during the first month of life in this infant and two others in whom the disease was suspected because of familial incidence. Two of the infants did not have phenylketonuria and their serum phenylalanine levels remained less than 10 mg. per cent.

The patient under discussion had a normal level at birth and serial studies showed a progressive rise reaching 60 mg. per cent at the end of the third week. At five days of age, with a serum phenylalanine level of 24 mg. per cent, the infant excreted none of the abnormal metabolites (o-hydroxyphenylacetic and p-hydroxyphenyllactic acids), but these did appear by the age of 16 days when the serum phenylalanine level was 47 mg. per cent. The infant did not excrete a detectable amount of phenylpyruvic acid until it was 34 days old. Treatment was started at this time.

The possible absence of phenylpyruvic acid from the urine of phenylketonuric infants before the age of one month calls for caution in arriving at a negative diagnosis based on testing during the first few weeks of life. During this age period determinations of the serum phenylalanine level should provide a more reliable test. These observations are consistent with the fact that some phenylketonuric children may develop normally for a few months before developing pathological symptoms, and they are consistent with the hypothesis that the excess phenylalanine is cleared through the placenta during intrauterine life. An alternative hypothesis which would also explain the delayed appearance of phenylketonuria is that phenylalanine oxidase is not present in newborn infants and that the production of phenylpyruvic acid, and the accompanying slight fall in serum phenylalanine levels, at about 1 month of age in this infant indicated the maturation of this enzyme system.

This patient had been under treatment for 7½ months at the time of the report of Horner *et al.* During this limited period of observation she had shown development within normal limits, and the most recent evaluation gave a development quotient of 100. Although further observations will be necessary, it seems reasonable to conclude from the data presently available that early treatment with a phenylalanine-low diet may prevent the development of mental retardation and the other psychological manifestations of this disease.

SODIUM AND POTASSIUM IN PERIODIC PARALYSIS

While familial periodic paralysis is a relatively rare clinical entity, it occurs with sufficient frequency so that work is being continued in the search for factors regarding both the mechanism involved in the induction of the paralytic attacks and the means of therapy. In the sporadic as well as the hereditary forms of this disorder, there are periods of transient acute, flaccid paralysis. According to M. R. Zigler and I. McQuarrie (*Metabolism* **1**, *116* (*1952*)) the onset of the disorder is commonest during the first decade of life, and the attacks become more serious and frequent during adolescence and early adult life.

In a typical attack, paralysis begins at the extremities and spreads to the muscles of the trunk. Deep reflexes in the involved muscles are either absent or markedly impaired during the period of paralysis. Other than mild apprehension, there appears to be no accompanying sensory or psychic disturbance. Death by suffocation may occur should the paralysis extend to the respiratory muscles. Paralysis tends to wear off gradually after a few hours, but there have been reports of rare instances where it lasted for one or two days. It has been noted that episodes of paralysis are most frequently induced by consumption of a diet especially rich in carbohydrate, and often a child who has this disorder may induce an attack of paralysis by overeating candy.

After it was found that there is an abnormality in potassium metabolism in these patients it was shown repeatedly that any change induced in the patient, which would cause a precipitous drop in the serum potassium (administration of glucose, insulin, over-dosing with DOCA, etc.), would tend to

induce paralysis. On the other hand, the administration of potassium salts causes a disappearance of the paralysis within 30 to 60 minutes if the material is given orally, and more promptly if it is administered intravenously.

J. W. Conn *et al.* (*Lancet* **I,** *802 (1957)*) have studied the role of sodium retention in triggering potassium sequestration in two young men with the familial type of periodic paralysis. Metabolic balance studies were carried out in both patients. The first patient was studied for eleven months, and the second subject, who was unrelated to the initial patient, was studied for 30 days in an effort to test, in another person, the metabolic concepts derived from the first study.

The subjects were maintained for different periods on one of two levels of sodium, 8 or 208 mEq per day. The basic diet was the same for either sodium intake, differing only by the addition of sodium chloride. The chloride ion was the only other variable being given at a level of 27 mEq per day on the low sodium diet and 229 mEq per day on the high sodium diet. In all the studies the potassium intake was kept constant at 132 mEq per day.

A complete paralysis, from the neck down, developed if the subjects who were being maintained on 208 mEq of sodium per day were given glucose and insulin. The paralytic attacks which occurred in both subjects lasted 24 to 48 hours. The administration of 1 mg. of 2-methyl-9-α-fluorohydrocortisone, which is an extremely potent sodium retainer, also induced this type of paralysis. When this steroid was given it was not necessary to administer glucose and insulin to provoke an attack. Whenever paralysis occurred there was a retention of sodium as shown by a positive sodium balance and an increase in the exchangeable sodium. In one of the subjects before administering the mineral corticoid the exchangeable sodium was 3,188 mEq and two days after administering this compound, when the paralysis was approaching its maximum, the total exchangeable sodium had increased to 3,690 mEq. During the time when the patient was developing a positive sodium balance the serum sodium rose and the serum potassium decreased to less than 2 mEq per liter. At the time that the serum potassium was decreasing the urinary output of potassium fell to less than 10 mEq per day. Under these conditions potassium was being transferred out of the vascular compartment and, in all probability, was moving intracellularly. Thus these individuals were sequestrating potassium in their body. In both cases during paralytic episodes the urinary and serum potassium fell sharply, but the retention of potassium was always preceded by a marked retention of sodium.

By inducing an episode of periodic paralysis with glucose and insulin a large retention of sodium occurred. This retention preceded the actual attack and was accompanied by a rapid rise in the aldosterone content of the urine (3 to 4 times that of normal). Following the episode of paralysis an intense diuresis of sodium occurs, during which time the urinary aldosterone falls well below normal (one-sixth of the pre-treatment level).

When the subjects were maintained on the low sodium diet (8 mEq per day) and were given 2-methyl-9-α-fluorohydrocortisone, there was neither a retention of sodium nor of potassium. The serum potassium did not fall and periodic paralysis did not occur. These subjects, while being maintained on the low sodium intake, had increased levels of aldosterone in the urine, yet they did not have attacks of paralysis. This led Conn and his associates to suggest that the sudden pooling of potassium, which accompanies attacks of periodic paralysis, is not due to increased amounts of aldosterone *per se*. But sodium must be available and must be retained by the body before potassium begins to be withdrawn from the extracellular fluid.

Samples of skeletal muscle taken during periods of high sodium intake were shown by analysis to contain excessive amounts of sodium and slightly subnormal concentrations of potassium. The sodium content of skeletal muscle ranged from 47 to 52 mEq

per kilogram on a wet weight basis, and the potassium varied from 75 to 85 mEq per kilogram of wet muscle. In the case of normal individuals, the sodium content has been reported to range from 21 to 31 mEq per kilogram of wet muscle and that of the potassium from 93 to 106 mEq per kilogram of wet tissue. Additionally, upon microscopic examination, characteristic lesions were noted in the muscle of patients with familial paralysis who were maintained on high sodium intakes. Numerous large and small glistening vacuoles were found within the muscle fibers. These vacuoles did not stain for fat or glycogen. On the other hand, skeletal muscle obtained from one of these subjects after 44 days on a low sodium diet did not show the characteristic vacuolar changes.

In regard to the clinical entity, periodic paralysis, there are several well known discrepancies which make it difficult to correlate the attacks of paralysis solely with the abnormality of potassium metabolism; a) the attacks may occur at serum potassium levels which, in other conditions, are not sufficient to cause muscular paralysis, b) in the same individual it is difficult to correlate the severity of attack with the level of potassium in the serum, c) when serial serum potassiums are drawn during an attack it was observed that muscular function returns while the serum potassium values are near the lowest obtained during the study, d) there are sporadic reports of instances where paralysis occurred without any decrease in serum potassium.

Conn and his co-workers suggest, in periodic paralysis, that the etiological common denominator is an excessive quantity of sodium within the cell. This does not eliminate low serum potassium levels or suddenly increased intracellular potassium as contributory factors. What is stressed, however, is that a retention of sodium is the primary factor which initiates the characteristic series of events leading to an attack of periodic paralysis. The important practical conclusion made by these investigators suggests that a diet low in sodium prevents attacks of periodic paralysis. To reduce the sodium in the body a preliminary de-salting with mercurial diuretics and/or carbonic anhydrase inhibitors, coupled with a low salt diet, may be required as an initial step.

ILEOSTOMY CHEMISTRY

In the course of therapy for certain disorders, such as ulcerative colitis, it is necessary to remove the large intestine and form an ileostomy (the proximal end of the small intestine is brought to the outside of the body as a permanent opening for the discharge of fecal contents). Since large amounts of fluid and electrolytes are lost through the ileostomy opening during the early postoperative period, it is often difficult to maintain the fluid and electrolyte balance of these patients. Recently N. Crawford and B. N. Brooke (*Lancet* **I,** *864* (1957)) studied the composition and volume of intestinal drainage from both new and established ileostomies. The amount of sodium, potassium, nitrogen, and calcium lost in the drainage was determined in eight patients with ileostomies.

Crawford and Brooke found that one of the characteristic features of most ileostomies is the large amount of watery fluid which is lost during the first six to ten days after the formation of the stomata. During the first postoperative week an average of two liters (range 1000 to 3000 ml.) of ileostomy drainage per day is lost. On the other hand, the average output of a well adapted ileostomy is approximately 200 to 500 ml. per day and the excreta has become semi-solid or formed. The mechanism for this fluid conservation in the case of the established ileostomy has not been determined.

Providing there were no complications,

losses of from 2 to 4 g. of nitrogen per day are found in the ileostomy drainage in the early period following operation. After the ileostomy becomes established the daily fecal nitrogen losses decrease to 1 to 2 g., which is within the range of that found in normal individuals. These investigators found by chromatography the following amino acids in the ileostomy fluid from two patients: glycine, alanine, valine, leucine, and arginine, but they did not believe that the losses of these amino acids were large enough to lead to a specific amino acid deficiency. A patient with severe enteritis and ileostomy diarrhea lost 8 g. of nitrogen per day at the peak of excretion. It should be noted that in spite of losing an average of 4 g. of nitrogen by way of the newly formed ileostomy, the patient with ulcerative colitis in a stage of exacerbation usually lost more nitrogen in the stool than he subsequently lost from an ileostomy. One male patient lost 11 g. of nitrogen in 24 hours in the stool before operation, whereas following operation the patient's losses were only 1 to 2 g. per day.

During the early period following an ileostomy, considerable amounts (200 to 400 mEq per liter) of sodium are lost. These investigators also noted that there was no pronounced renal conservation in spite of these large extrarenal sodium losses. Following the adaption of the ileostomy there was a thickening of the excretions and the fecal concentration of sodium decreased to approximately 150 mEq per liter. The loss of sodium in the immediate period following an ileostomy is great for two reasons; first, the volume of drainage is large, and secondly, the concentration of sodium per liter of ileostomy fluid is high (200 to 400 mEq).

The high concentration of sodium (200 to 400 mEq) reported in the ileostomy drainage by these investigators would make the fluid very hypertonic as compared to other body fluids. On the other hand J. S. Lockwood and H. T. Randall (*N. Y. Acad. Med.* **25,** *228 (1949)*) determined the sodium content of ileostomy drainage from a group of patients immediately after ileostomy, and from one individual after the ileostomy had become adapted. They noted that an average of 129 mEq of sodium per liter was being lost with the range of 105 to 143 in the newly formed ileostomies. The adapted ileostomy drainage had a sodium content of 46 mEq per liter. The discrepancies between the data obtained by these two groups cannot be resolved definitely at present.

Following ileostomy the patients usually lose about 10 mEq of potassium per liter of drainage, or roughly 20 mEq per day. This rate is continued after the ileostomy has been established. Two of the patients were losing large amounts of potassium preoperatively, 35 and 85 mEq per day, and following the ileostomy the losses diminished to about 10 mEq per day.

In the early period after an ileostomy the amount of calcium in the drainage fluctuated between 40 to 100 mEq per day (800 to 2000 mg.). But with the decreased volume of the drainage which occurred after the ileostomy had been established for several weeks, there was a diminution of the calcium loss. This, the authors believed, would have been greater except for the fact that there was an increased calcium intake associated with the more liberal diet that the patients were consuming.

The qualitative and quantitative data presented by these investigators have considerable importance in attempting to supply adequate fluid and electrolyte replacement in patients who have had ileostomies, particularly during the early stages before the ileostomy has become adapted. By knowing the volume of fluid lost and its composition it should be easy to administer, either orally or intravenously, sufficient amounts of fluid and electrolytes to cover the predicted losses. Patients with ileostomies which have not yet become adapted can develop large sodium and fluid deficits which may lead to sudden circulatory collapse because of the decreased plasma and extracellular fluid volume. However, by occasionally determining the value of sodium in the ileostomy

drainage and knowing the daily loss of such fluid, the calculation and administration of adequate fluid and electrolytes becomes simple. Thus it need not be necessary to conduct elaborate balance studies, or to determine frequently serum electrolyte concentrations, in order to evaluate the status of fluid and electrolytes in such individuals.

THE DUMPING SYNDROME: SOME CURRENT ETIOLOGIC CONCEPTS AND DIETARY MANAGEMENT

The dumping syndrome is a fairly frequent and disturbing side effect which has been known to occur after any type of gastrectomy.

The development of this syndrome has been attributed in turn to rapid emptying with distention of the efferent jejunal loop, to stretching of the afferent jejunal loop by stasis of biliary and pancreatic secretion, and to hypoglycemia. None of these, however, seemed to fit in with the classical and unmistakable description of the dumping syndrome.

Recently more attention has been directed toward the changes in plasma volume and serum electrolytes occurring simultaneously with the clinical picture of this syndrome.

A recent review and evaluation of the etiologic concepts of this clinical entity was made by B. M. Webber, *et al.* (*New Engl. J. Med.* **256,** *285* (*1957*)). They present a study conducted on 16 postgastrectomy patients, eight of whom had symptoms of the dumping syndrome of varying severity.

Each patient was given 150 ml. of 50 per cent glucose orally. Following this, plasma volume and serum potassium were checked. Plasma volume was determined in 11 patients in this group, according to the isotope dilution method using radioactive iodinated serum albumin. This determination was done at the onset of clinical symptoms in six patients who had the dumping syndrome, and at 15 to 20 minutes after the test meal in the five patients who were asymptomatic. No patient without history of clinical dumping syndrome manifested this syndrome after the ingestion of the hypertonic glucose solution.

Ten of the 11 patients exhibited a decrease in plasma volume. In the asymptomatic patients, the range was 0 to 300 ml., with an average decrease of 194 ml. In those patients who exhibited the syndrome, the range was 31 to 405 ml., with an average decrease of 150 ml. There was obviously no correlation between the magnitude of the decrease in plasma volume and the severity, or even the occurrence at all, of symptoms. There is a discrepancy between these results and those of K. E. Roberts *et al.* (*J. Clin. Invest.* **32,** *597* (*1953*)) who reported a fall in plasma volume ranging around 500 ml., using the Evans blue dye dilution method.

Serial serum potassium determinations were determined in the whole group of patients, and 13 of 16 revealed a maximal fall in serum potassium of 0.5 to 1.5 mEq per liter, occurring 30 to 60 minutes following the ingestion of the glucose solution. The incidence, timing, and magnitude of this change appeared to bear no correlation with the occurrence of the dumping syndrome.

The authors conclude, on the basis of their study, that the described changes in plasma volume and serum potassium are not causative factors in the production of this syndrome, but that they are merely physiological changes resulting from the gastrectomy.

In the realm of management of this distressing disturbance, on the other hand, definite progress has been achieved. A program of dietary management is described by F. W. Robinson and A. C. Pittman (*Surg. Gynec. Obstet.* **104,** *529* (*1957*)). This comprises a diet essentially high in protein and fat and low in carbohydrates, with complete avoidance of simple sugars. They

advocate the application of this regimen to all postgastrectomy patients, starting during convalescence and gradually increasing the diet in three steps which they describe, complex carbohydrates being added last and in limited amounts. This diet should be followed permanently. In the authors' experience, a significant improvement followed the institution of this regimen in 80 per cent of the group studied.

LYSINE REQUIREMENTS OF MEN AND WOMEN

The extensive work of Rose and his associates upon the amino acid requirements of young men has been the subject of numerous previous reviews (*Nutrition Reviews* **14,** *232* (*1956*)). More recently, the work of Leverton *et al.* (*Ibid.* **14,** *269* (*1956*)) on the amino acid requirements of young women was reviewed. Lysine, one of the essential amino acids of much current interest, was not studied, by Leverton *et al.* E. M. Jones, C. A. Bauman and M. S. Reynolds (*J. Nutrition* **60,** *549* (*1956*)) and H. E. Clark, E. T. Metz and co-workers (*Ibid.* **62,** *71* (*1957*)) have now provided additional data upon the human requirement of this essential amino acid.

The study of Jones *et al.* utilized women between 19 and 34 years of age who maintained their usual academic pursuits during the study. They were first fed a normal diet containing 10 g. of nitrogen and 4 g. of lysine (by analysis) to establish that they could maintain balance and to adjust them to the level of nitrogen intake used in the experimental periods. They were fed a semisynthetic diet containing a few low protein foods similar to that used by Leverton *et al.* (*Nutrition Reviews* **14,** *269* (*1956*)). Purified essential amino acids supplied approximately 2 g. of nitrogen, 4 g. of glycine, and 4 g. of diammonium citrate. In the experimental periods varying levels of lysine were substituted for glycine. Fourteen subjects were studied at two or more levels of lysine intake, some at six different levels between 0.10 and 1.60 g. per day. The data showed considerable variation among the subjects when they were receiving the same levels of lysine. Also, repeated balance studies at the same level upon the same subject demonstrated a considerable change from one period to another. Such variations are familiar to workers in this field. Nevertheless the data clearly showed a trend toward more positive balance as the amount of lysine was increased.

The mean balance upon all of the subjects was essentially zero at levels of intake between 0.4 and 0.6 g. per day, which is the estimated requirement. Two subjects showed an appreciable negative balance at an intake of 0.4 g. of lysine, but both attained balance when the level was raised to 0.5 g. These values are similar to the estimated requirements of men given by Rose *et al.* (*J. Biol. Chem.* **214,** *579* (*1955*)) which were 0.4, 0.4, 0.6, 0.7, 0.7 and 0.8 g. per day. As in the studies of Leverton *et al.*, the tendency toward somewhat lower requirements than obtained by Rose may be due in part to the smaller size of the women used as subjects. However, no correlation was found between the apparent lysine need and body size in these studies.

The data of Clark *et al.* are less satisfactorily presented and more difficult to evaluate. The diet contained 9 g. of nitrogen and approximately half of this was supplied by wheat flour, corn meal and a few other low protein foods. The remainder was supplied by purified amino acids and ammonium citrate. Sufficient of the essential amino acids, except lysine, was supplied to provide the amounts found in 20 g. of egg protein. This is similar to the amount provided by Jones *et al.* and is more than the minimum needs estimated by Rose and Leverton. The unsupplemented diet provided between 470 and 510 g. of lysine per day.

The authors summarize the amounts of lysine required to permit equilibrium in the

ten subjects (five men and five women between the ages of 23 and 29 years). "A subject was judged to be in equilibrium when nitrogen balance was at or near zero." These values range from 0.5 to 0.9 g. per day. At these intakes the balance ranged from −0.03 to +0.30. The authors also found, contrary to Rose *et al.* and Jones *et al.*, that there was a significant correlation between body weight, surface area, and metabolic body size to lysine need. Thus, the data show that balance was achieved at these levels of lysine intake. They do not permit an independent estimate of the validity of the values taken as the minimum need.

Rose *et al.* (*J. Biol. Chem.* **210,** *331* (*1954*)) have emphasized the greater caloric need of subjects fed the synthetic diets containing purified amino acids. In these studies Jones *et al.* report that upon the normal diet from 28 to 36 calories per kilogram per day, mean 32.8, were required for maintenance of weight. Upon the simplified diet the values ranged from 33 to 43, mean 37.6, apparently a slight increase. On the less purified diets used by Clark *et al.*, the men maintained weight on 45 to 58 calories per kilogram and women upon 29 to 50 calories per kilogram. They comment that these do not appear excessive compared to the estimates of the Food and Nutrition Board of 49 for men and 42 for women.

The diets of women studied by Futrell *et al.* (*J. Nutrition* **46,** *299* (*1952*)) and Reynolds *et al.* (*J. Am. Dietet. Assn.* **29,** *359* (*1953*)) contained 1.7 to 8.6 g. of lysine. Block and Bolling (The Amino Acid Composition of Protein and Foods, C. C. Thomas Publishing Co., Springfield (1951)) have estimated the "average" American diet to contain about 5 g. daily. The diet used by Clark *et al.* contained 159 g. of white flour and 21 g. of corn meal. These amounts supplied over 90 per cent of the lysine in the basal diet, yet this diet maintained balance in some of the subjects. As has been previously pointed out (*Nutrition Reviews* **14,** *269* (*1956*)) and emphasized by Rose (*J. Biol. Chem.* **211,** *815* (*1954*)), minimum estimates must be interpreted with caution and diets should contain some undefined amount in excess of minimum need. Also, the utilization of the balance method as an estimate of requirement has various disadvantages.

DIET AND SALMONELLA INFECTION IN CHICKS

The studies of H. A. Schneider and L. T. Webster (*J. Exp. Med.* **81,** *359* (*1945*)) demonstrated that diet played an important role in the resistance of mice to *Salmonella enteritidis* infection. In a more recent study it was observed that young chickens could be protected to a considerable extent against *Salmonella gallinarum* infection by high-vitamin supplementation of the diet (*Nutrition Reviews* **14,** *188*, *214* (*1956*)).

In a preliminary study H. W. Smith observed that the dietary level of certain protein concentrates affected the mortality rate, as well as the infection rate, of chicks infected with *S. gallinarum* (*Brit. J. Exp. Path.* **35,** *447* (*1955*)). A recent paper describes a more thorough study of the dietary protein relationship to this infection (H. W. Smith and L. G. Chubb, *J. Comp. Path.* **67,** *10* (*1957*)).

Day-old chicks were reared to five weeks of age on practical rations that were nutritionally complete. They were then transferred to the experimental diets for three weeks, at which time they were infected orally with *S. gallinarum*.

When the experimental diet consisted of finely ground wheat alone, 62 of 75 chicks died of the infection within 17 days. Supplementation with 2.5 per cent of fish meal reduced the fatality to only 39 of 75 chicks. Increasing the fish meal to 10 or 40 per cent again resulted in high mortality rate. Peanut meal supplementation (50 per cent level) likewise seemed to increase the mortality rate over that on the basal wheat diet.

The complex relationship of diet to infection is further emphasized by the results obtained when diet changes were made three days before the chicks were infected. The disease was much less severe in a group of chicks whose diet was changed from ground wheat, plus 40 per cent of fish meal, to ground wheat only (30 per cent mortality), than it was in those fed either ground wheat throughout the experiment or ground wheat plus 40 per cent of fish meal (70 and 88 per cent mortality, respectively). Feeding 10 per cent supplementary fish meal throughout resulted in more severe infection (57 per cent mortality) than when the fish meal was deleted three days before infecting (27 per cent mortality).

The two different, or opposite, effects obtained with the diet of ground wheat only, depending upon whether it was fed throughout or whether it was substituted for a high-protein diet three days before infecting, is difficult to explain. As the authors suggest, it is possible that the high fish meal diets place a stress upon the liver in addition to supplying an essential anti-infective factor, and that the carry-over of the protective factor is sufficient to express itself following removal of the high-fish meal stress condition.

Smith and Chubb also investigated the effect of starvation on *S. gallinarum* infection and failed to note any adverse effects. They therefore conclude that the theory advanced by R. J. Dubois (*Lancet* **I,** *1003 (1955)*) may not have wide application. Dubois found that the course of bacterial diseases produced by the parenteral route in mice was more severe in animals that had been starved before infection than in animals that had been fed normally.

Smith and Chubb recognize that the amino acid balance in a diet of wheat alone is probably far from ideal with respect to requirements for optimum health in growing chicks. It is possible that the most protective diet, the one supplemented with 2.5 per cent of fish meal, came nearest to providing an ideal amino acid balance.

These results again emphasize that the disease resistance mechanism is a complex one and that dietary responses are often difficult to interpret, particularly when diets are used which are difficult to define from the standpoint of their nutrient composition. This in itself, however, is not a valid argument against the use of diets composed of natural products in studies of dietary influences on disease, since, under field conditions, these same practical disease problems prevail.

SALT IN SHEEP RATIONS

The practice of providing livestock with a salt supplement is common. However, with regard to sheep, there is little scientific evidence of any benefit from salt licks either in mixed rations or under grazing conditions.

Workers at the Veterinary Research Station in New South Wales have recently noted a rather striking benefit from sodium chloride supplementation of a fattening ration for sheep (G. L. McClymont, K. N. Wynne, P. K. Briggs and M. C. Franklin, *Aust. J. Agr. Res.* **8,** *83 (1957)*).

The rations used were (1) 100 per cent oat grain; (2) 50 per cent each of oat grain and alfalfa meal; (3) 75 per cent of oat grain and 25 per cent of alfalfa meal; (4) 50 per cent each of oat grain and wheat; and (5) 75 per cent of oats and 25 per cent of wheat. The oat and wheat rations were supplemented with 1.5 per cent of limestone to correct the calcium deficiency.

On all rations the addition of 0.25 per cent of sodium chloride resulted in a growth stimulation. The growth response obtained ranged from 19 to 58 per cent over that observed on the corresponding rations without the salt addition. The basal rations contained from 0.009 to 0.062 per cent of

sodium and from 0.05 to 0.42 per cent of chlorine.

In order to rule out the possibility that the growth response was not due to the chloride, a second experiment was conducted in which the growth response to sodium chloride was compared with that obtained with sodium bicarbonate. The latter was fully as effective as the former.

The authors conclude that the sodium requirement of the sheep was in excess of 0.06 per cent of the diet, or 0.88 g. per day.

Food consumption was noted to increase on four of the five rations when salt was added, suggesting that under these conditions the palatability of the ration was improved by the salt. On the fifth diet however, a 37 per cent increase in growth was noted from the salt addition, while feed intake was not increased. On all diets the salt supplement appreciably increased the efficiency of utilization of the consumed feed.

These results confirm the observation of workers at the Kansas Agricultural Experiment Station, Manhattan, who noted growth response from sodium chloride in lambs fed a ration of corn and alfalfa (E. L. Hix, T. D. Bell, D. B. Parrish and A. L. Good, *Kans. Agr. Exp. Sta. Circular* **273,** (*1951*); **283** (*1952*)).

The Kansas group also noted that the digestibility of all ration components was reduced 3 to 4 per cent, or more, when salt was withheld.

It is of interest that both groups of workers noted that serum sodium levels remained essentially constant and at normal levels despite the severe deficiency of sodium in the diet. With extended periods of sodium deficiency the blood potassium levels tended to increase. Further imbalance in the dietary sodium to potassium ratio by the addition of potassium bicarbonate was noted by the Kansas workers to create a circulatory deficiency of sodium and a severe dehydration.

In earlier studies with chicks it was noted that both sodium and potassium appeared to be toxic if the other were present in very small amounts (*Nutrition Reviews* **12,** *77* (*1954*)).

These results would tend to support the common belief that farm animals require salt to overcome the antagonistic effects of the high potassium content of animal feeds.

VITAMIN B_{12} CONTENT OF CHEESE

Information on vitamin B_{12} distribution in food is still rather sparse. It is known, in general, that the only source of vitamin B_{12} in the usual American diet is food of animal origin, such as meat, milk, eggs, and cheese. Fruits and vegetables are devoid of vitamin B_{12} as far as is known (*Nutrition Reviews* **7,** *210* (*1949*); **13,** *113* (*1955*); **14,** *79* (*1956*)).

The vitamin B_{12} potencies of Cheddar, Swiss, and Cottage cheese have been studied in detail by A. M. Hartman, L. P. Dryden, and R. E. Hargrove (*Food Res.* **21,** *540* (*1956*)). Both four-week rat assays and microbiological assays (*Lactobacillus leichmannii*) were used for determining vitamin B_{12}.

As measured by rat assay, Cheddar and process Swiss cheese had about the same potency, an average of 2.1 and 1.8 micrograms of vitamin B_{12} per 100 g. of cheese on a fresh basis. Process Cheddar cheese was somewhat less potent (1.2 micrograms per 100 g.), whereas natural Swiss cheese contained 3.6 micrograms of vitamin B_{12} per 100 g. on a fresh basis. The greater potency of the Swiss cheese is probably due to the synthesis of vitamin B_{12} by propionic acid bacteria, as suggested by the authors. Cottage cheese was found to contain 0.8 micrograms of vitamin B_{12} per 100 g. (which amounts to 3.8 micrograms per 100 g. on a dry basis). This is a significant amount.

Except for Cottage cheese, values obtained with the microbiological assay aver-

aged only about half of the rat assay results in spite of attempts to liberate additional vitamin B_{12} activity by various treatments. The reason for these differences between the rat and microbiological assays could not be determined. However, the authors suggested that either vitamin B_{12} was unavailable to the microorganism for some reason or was destroyed in preparing the cheese samples for microbiological assay. Evidence was presented that the rat growth test did not respond to substances in cheese other than vitamin B_{12} (although results with methionine were not given).

Studies were made of the vitamin B_{12} potency of Cheddar cheese samples made from one original batch of milk after various periods of curing at different temperatures. No significant differences were found in the vitamin B_{12} potency of the original curd as compared with cheese as old as eighteen months. Also, the vitamin B_{12} level was not affected by temperature of storing (40° F. or 55° F.).

The above values are in good agreement with those obtained by U. J. Lewis, U. D. Register, H. T. Thompson, and C. A. Elvehjem (*Proc. Soc. Exp. Biol. Med.* **72,** *479 (1949)*) and by M. Hietaranta and M. Antila (*Proc. XIII Intern. Dairy Congress* **3,** *1436 (1953)*) for Cheddar cheese and Swiss cheese, respectively. The values compare favorably with the vitamin B_{12} content of milk (Lewis and co-workers, *Loc cit.*; A. M. Hartman, L. P. Dryden, and G. H. Riedel, *J. Nutrition* **59,** *77 (1956)*) and with other good sources of vitamin B_{12} (*Nutrition Reviews* **13,** *113 (1955)*).

Although the vitamin B_{12} requirement of man is not known, Cheddar cheese, Swiss cheese, and Cottage cheese may be considered good sources of this vitamin. Information of this nature is useful in the planning of diets.

GLYCEROL METABOLISM AND FAT ABSORPTION

Glycerol represents between 10 per cent (for tristearin) and 30 per cent (for tributyrin) of administered triglycerides. Therefore, pathways different from those normally associated with fat metabolism may be important in the metabolism of this fraction of dietary fat. An investigation of the metabolism of glyceride-glycerol by L. I. Gidez and M. L. Karnovsky (*J. Biol. Chem.* **223,** *293 (1956)*) provides some information, not only about the fate of the glycerol moiety of ingested glycerides, but also about fat digestion and absorption, and represents an extension of their previous study on the metabolism of free glycerol (*J. Biol. Chem.* **206,** *229 (1954)*) and of that of the study reported in *Nutrition Reviews* **12,** *122 (1954)*.

Young male rats which had been fasted were given, by stomach tube, triolein or tributyrin labeled with C^{14} in the glycerol moiety. They were placed in metabolism cages and were killed at intervals up to eight hours after the administration of the labeled fat. The gastrointestinal contents, blood, liver, stomach, and small and large intestines were removed and radioactivity in various lipid fractions, in liver glycogen, and in expired carbon dioxide was measured.

Both of the administered triglycerides were rapidly absorbed, 95 per cent having disappeared from the intestinal lumen within six hours. The quantities administered were relatively small (of the order of 225 mg. for a rat weighing about 160 g.) which might account for the short time required for complete absorption. Triolein was absorbed rapidly during the first hour, relatively slowly between one and two hours, and more rapidly again between two and four hours after administration. This suggested that when the mucosal cells were loaded with fat, entry of further fat was prevented until that previously absorbed was further metabolized or was transported to other tissues.

From the measurement of CO_2 excretion it was evident that about 20 per cent of the glycerol from triolein was oxidized within four hours. This compared with approximately 40 per cent of an equivalent amount of free glycerol. On the assumption that complete hydrolysis of glyceride preceded oxidation of glycerol, it was possible to estimate the extent of lipolysis from the relative rates at which free glycerol and glyceride-glycerol were oxidized. These calculations indicated that although 75 per cent of the fat was absorbed between one and two hours, only 12 per cent was completely hydrolyzed. This latter figure rose to about 50 per cent after four hours and almost 100 per cent at eight hours. These figures are only approximate because of the indirect measurements on which they are based and because many factors, such as the rate of liberation of the hydrolytic products and the use of the released glycerol in synthetic reactions, cannot be taken into account.

There was a rapid fall in the radioactivity of the neutral fat in the intestinal wall between two and four hours, which suggested that a substantial amount of glyceride-glycerol was released through hydrolysis of fat in the intestinal mucosa up to four hours. Hydrolysis at extra-intestinal sites appeared to be more significant after four hours. Some free glycerol was recovered from the intestinal lumen indicating that some complete hydrolysis of triglycerides had occurred prior to absorption. This represented a relatively small fraction (*Nutrition Reviews* **14,** *88, 308 (1956)*).

Measurement of the radioactivity of the neutral fat in the intestinal wall indicated that between 30 and 40 per cent of the absorbed fat was in the intestinal wall between one and two hours after ingestion. By four hours, this had dropped to less than 5 per cent. Acetone fractionation indicated that neutral fats were four times as active as the phosphatides, per mg. of carbon. There was, however, a significant amount of radioactivity in the phosphatide fraction. A comparison of the radioactivity of intestinal, plasma and liver lipids over the eight-hour period showed that the intestinal lipids contained the greatest amount of activity up to four hours. There was considerably less in the serum lipids, and despite the fact that free glycerol had previously been shown to enter the liver lipid fraction readily, relatively little radioactivity was detected at any time in the liver lipids. The ratios of phosphatide activity to neutral fat activity were almost the same for intestine and liver. Very little radioactivity was present in liver glycogen.

Measurement of the radioactivity of the phosphatides showed that labeled phosphatides were present in the intestinal lumen, in the intestinal mucosa and in the liver. Since free glycerol is not readily incorporated into intestinal phosphatides (M. Buensod, P. Favarger, R. A. Collet, *Helv. Physiol. and Pharmacol. acta* **11,** *45 (1953)*), and since only small amounts of absorbed free fatty acids are incorporated into lymph phosphatides (*Nutrition Reviews* **9,** *212 (1951)*), the observation that 8.4 per cent of the labeled glycerol was present in the intestinal phosphatides would support the hypothesis that the triglycerides, as such, might be precursors of phosphatides (A. Reiser, and J. W. Dieckert, *Proc. Soc. Exp. Biol. Med.* **87,** *622 (1954)*).

These observations, in the main, support the views of Frazer that total lipolysis is not a prerequisite of absorption (*Nutrition Reviews* **10,** *193 (1952)*). They do, on the other hand, indicate that total lipolysis may occur (B. Borgstrom, *Acta Physiol. Scandinav.* **25,** *328 (1952)*). They suggest an initial rapid phase of fat absorption and a second slow phase followed by more rapid absorption again. They also indicate that glycerol is released quite rapidly from triglycerides and is rapidly oxidized to carbon dioxide, and that, under the conditions of this experiment, there was little synthesis of either glycogen or liver triglycerides from triglyceride glycerol.

These isotopic experiments on triglyceride

metabolism offer confirmation of many previous observations on fat digestion and absorption and suggest a number of fruitful lines of investigation in this field. Two of these, in particular, merit further study; the possibility that triglycerides may be directly converted to phosphatides, and the possibility that glycerol in the free form may be metabolized differently from triglyceride-glycerol. The authors recognize that the metabolism of triglycerides fed alone to starved animals may differ considerably from that of triglycerides fed as part of a complete diet, and acknowledge that similar studies on animals receiving adequate diets are essential.

INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

Whatever the basic cause of atherosclerosis, hypercholesterolemia is often tacitly accepted as a predisposing, or contributory, factor in its pathogenesis. With increasing knowledge, it may develop that treatment of hypercholesterolemia per se represents little more than treatment of a symptom, but at present such treatment provides one of the few available approaches to the understanding and cure of this disease.

On the basis of present knowledge, serum cholesterol levels are most likely to be depressed in the following ways: (1) alteration in dietary intake of metabolites affecting serum cholesterol levels (A. Keys *et al.*, *Clin. Chemistry* **4,** *34 (1955)*; *Nutrition Reviews* **13,** *8, 44, 138 (1955)*); (2) alteration in partition of cholesterol between tissues and blood; (3) inhibition of endogenous cholesterol synthesis.

The last mentioned approach received little attention until D. Steinberg and D. S. Fredrickson (*Ann. N. Y. Acad. Sci.* **64,** *579 (1956)*) carried out a series of investigations oriented in this direction. These authors calculated that since the average daily dietary intake of cholesterol by man is about 0.5 g., and since 1.5 g. of cholesterol is turned over per day (I. M. London and D. Rittenberg, *J. Biol. Chem.* **184,** *687 (1950)*), at least 1 g. must be contributed daily by endogenous synthesis. If it were possible to reduce the rate of synthesis without change in dietary intake, and if the decreased rate of synthesis were not compensated by a decrease in rate of degradation, the result would be equal to a removal of about 0.1 g. of cholesterol daily from the total pool. In four weeks the decrease in the serum would be 29 per cent unless the deficiency were relieved in some way.

That homeostatic mechanisms do operate is well known. For example, the rate of synthesis of cholesterol has been shown to depend upon the level of cholesterol in the liver (R. G. Gould, *Am. J. Med.* **11,** *209 (1951)*). Other controls seem to operate at the point where cholesterol is incorporated into lipoproteins and where these lipoproteins are introduced into, and removed from, the serum. In any case it appears that endogenous synthesis normally adds considerably more cholesterol to the body pools than does the diet. An attempt to reduce this contribution by the feeding of appropriate inhibitory substances seems to merit investigation, especially since the pathway of biosynthesis of cholesterol has recently been outlined in some detail (J. L. Rabinowitz, F. Diture, F. Cobey, and S. Gurin, *Fed. Proc.* **14,** *760 (1955)*; R. B. Clayton and K. Bloch, *J. Biol. Chem.* **281,** *319 (1956)*).

A partial outline of the probable pathway for cholesterol biosynthesis would include the following steps: (1) activation of free acetate; (2) formation of acetoacetate; (3) formation of a series of branched chain acids; (4) formation of squalene; (5) transformation of squalene to cholesterol.

J. Cottet *et al.* (*Bull Acad. Natl. Med., Paris* **25–27,** *441 (1953)*) have reported significant decreases in serum cholesterol of normal rats and hypercholesterolemic patients fed the sodium salt of α-phenylbutyric

acid. This compound can be considered as a type of analog of a five-carbon branched-chain acid in which the phenyl group replaces a methyl group. Steinberg and Fredrickson (*Loc. cit.*) undertook to explore the possibility that this preparation might inhibit cholesterol biosynthesis at step three or four above. *In vitro* studies with rat liver slices showed that this compound did indeed inhibit the incorporation of 1-C^{14} acetate into cholesterol.

It became apparent, however, that α-phenylbutyrate was not acting as a branched-chain acid analog, since incorporation of 1-C^{14} acetate into fatty acids and acetoacetate was inhibited to about the same extent, as was incorporation into cholesterol. This suggested that the site of action was at an early step common to both cholesterol and fatty acid synthesis. According to the authors, this step could be either the activation of acetate to form acetyl coenzyme A, or the formation of acetoacetate. The studies of J. Avigan, J. H. Quastel and P. G. Scholefield (*Biochem. J.* **60,** *329* (*1955*)) on the effect of other aromatic acids make it appear probable that the rate of formation of acetoacetate is altered.

Sodium β-phenylvalerate was studied by Steinberg and Fredrickson (*Loc. cit.*) in a similar manner and was also found to inhibit at steps (1) or (2) in the path of cholesterol biosynthesis outlined above. *In vivo* studies were undertaken in the rat by these same authors. Alphaphenylbutyrate was added to the food at a level giving a daily intake of 150 to 200 mg. per kg. After seven weeks of this treatment, each animal was given 25 microcuries of 1-C^{14} acetate intraperitoneally two hours prior to sacrifice. There was no significant difference in serum cholesterol levels between this experimental group and a control group. The treated group did, however, display a 25 per cent inhibition in the rate of C^{14} incorporation into liver cholesterol. The authors consider that this paradox (decreased rate of cholesterol synthesis without decline in serum cholesterol level) might be due to the possibility that inhibition of acetate activation would not necessarily influence the net conversion of the primary metabolic building blocks into cholesterol, but it would decrease the rate of incorporation of free acetate into cholesterol.

The authors collaborated with R. Masters in an experiment (Steinberg and Fredrickson, *Loc. cit.*) which showed that acetate activation is indeed inhibited by α-phenylbutyrate. Using a partially purified preparation of acetyl CoA kinase complex from yeast, they observed a 69 per cent inhibition in the rate of acetyl coenzyme-A formation in the presence of 10^{-2} M sodium phenylbutyrate. Attempts to duplicate the clinical results reported by Cottet *et al.* (*Loc. cit.*) which included lowering of serum cholesterol by as much as 40 per cent after six weeks of treatment with β-phenylbutyrate, resulted in failure.

G. M. Tomkins, H. Sheppard, and I. L. Chaikoff (*J. Biol. Chem.* **293,** *781* (*1953*)) have reported that the feeding of several steroids in large doses depressed the incorporation of acetate into the liver cholesterol of rats. Steinberg and Fredrickson (*Loc. cit.*) were of the opinion that of these compounds, 4-cholestenone would be of special value for study since it apparently cannot be converted to cholesterol (G. M. Tomkins *et al.*, *Loc. cit.*) and may act as an analog of cholesterol in the later stages of cholesterol synthesis, thus inhibiting reactions somewhere between squalene and cholesterol. Results of experiments utilizing 1 per cent of this compound in the diet of rats included a dramatic inhibition of cholesterol synthesis with no effect on carbon dioxide production. In addition there was a smaller but significant suppression of fatty acid synthesis, but no inhibition of acetoacetate formation. The serum cholesterol level in these animals was 44 per cent below that of control animals. A striking and disturbing finding at autopsy was a remarkable hypertrophy of the adrenal glands in all of the experimental animals. In a first group, killed 40 days after the start of 4-cholestenone feeding, these glands were approximately four times the normal size.

After eight weeks on the supplemented diet, 22 per cent of the animals had died and weight loss had become marked. The authors speculate that the hypertrophy of the adrenal glands might be due to a block in the normal production of adrenal steroids, for which cholesterol has been shown to be an important precursor. Analyses of the glands showed a reduction in cholesterol concentration and an accumulation of an unsaponifiable lipid. Analyses of the livers of these animals revealed a marked increase in the total non-saponifiable lipid but no significant change in the level of cholesterol itself. The properties of the extra non-saponifiable lipid in these livers suggest that it is hydrocholesterol.

Although the above high level of feeding was obviously not a clinically useful method of reducing the level of cholesterol in serum, preliminary studies in which rats were fed smaller amounts of 4-cholestenone indicate that blood cholesterol may be reduced without impairing normal growth and without causing adrenal hypertrophy. Before clinical applications can be considered, the metabolism of the end product dihydrocholesterol must be closely investigated.

METHYLATION AND DEHYDROXYLATION OF HYDROXYAROMATIC COMPOUNDS

The ability of the animal body to introduce one or more hydroxyl groups into aromatic compounds is well known. The subsequent conversions of the hydroxyaromatic compounds to glycine, glucuronide, and ethereal sulfate derivatives are equally well known and are summarized in text books under the heading of detoxification mechanisms. As late as 1949 E. Lederer (*J. Chem. Soc.* **part 3,** *2119 (1949)*) stated that "Methylations of phenolic hydroxyl groups have not been observed in the animal body". N. F. Maclagan, and J. H. Wilkinson (*Nature* **168,** *251 (1951)*) reported the methylation of a hydroxyaromatic compound in the human body. A. N. Booth, C. W. Murray, F. T. Jones and F. DeEds (*Fed. Proc.* **14,** *321 (1955)*; *J. Biol. Chem.* **223,** *251 (1956)*), and DeEds, Booth and Jones (*Fed. Proc.* **14,** *332 (1955)*; *J. Biol. Chem.* **225,** *615 (1957)*) reported on the ability of rats and rabbits to methylate the number 3 hydroxyl group of protocatechuic acid, 3,4-dihydroxyphenylacetic acid, and caffeic acid to yield vanillic, homovanillic, and ferulic acids, respectively.

These observations by DeEds, Booth and Jones on methylation of phenolic hydroxyl groups grew out of studies on the absorption and metabolism of rutin and quercetin, in which it was demonstrated that the flavonoid molecule suffered cleavage to yield 3,4-dihydroxyphenylacetic acid. These workers not only demonstrated that part of the 3,4-dihydroxyphenylacetic acid was methylated, but also that another portion was dehydroxylated in the 4-position to yield meta-hydroxyphenylacetic acid. They extended their observations on the metabolic fate of caffeic acid and showed that, in addition to the formation of ferulic acid by methylation, dehydroxylation also occurred and yielded meta-hydroxyphenylpropionic acid. It is also significant, as shown by these investigators, that the newly discovered biochemical mechanisms of methylation and dehydroxylation of phenolic hydroxyl groups are not limited to compounds of dietary origin but are applicable to certain body constituents such as DOPA which Kuo-Huang, Lin (*J. Formosan Med. Assn.* **47,** *1 (1948)*), DeEds, Booth and Jones (*Fed. Proc.* **14,** *332 (1955)*), Chr. V. Euler, U. S. Euler, and I. Floding (*Acta physiol. Scandinav.* **33,** *suppl. 118, 32 (1955)*) and Euler (*Ibid.* **33,** *suppl. 118, 39 (1955)*) have shown gives rise to 3,4-dihydroxyphenylacetic acid, a precursor of meta-hydroxyphenylacetic and homovanillic acid. The formation of homovanillic acid from DOPA was confirmed by K. N. F.

Shaw, A. McMillan and D. M. Armstrong (*Fed. Proc.* **15,** *353* (*1956*)), but they did not confirm the production of meta-hydroxyphenylacetic acid by dehydroxylation. However, this biochemical reaction has again been demonstrated by Booth, Jones and DeEds (*Ibid.* **15,** *223* (*1956*)) in studies on the metabolic fate of the flavanone homoeriodictyol, which has a methoxy group in the 3′-position and a hydroxyl group in the 4′-position. Oral administration of this flavanone to rabbits resulted in the urinary excretion of meta-hydroxyphenylpropionic acid. To account for this there must have been dehydroxylation at the 4′-carbon and demethylation of the methoxy group on the 3′-carbon before, or after, splitting of the homoeriodictyol molecule.

More recently these biochemical reactions of methylation and dehydroxylation of hydroxyaromatic compounds have received additional confirmation. J. M. Price and L. W. Dodge (*J. Biol. Chem.* **223,** *699* (*1956*)) demonstrated the presence of the 8-methyl-ether of xanthurenic acid in normal human urine as a result of methylation of xanthurenic acid. The dehydroxylation of kynurenic acid to form quinaldic acid has now been reported by H. Takahashi, M. Kaihara and Price (*Ibid.* **223,** *705* (*1956*)). Takahashi and Price (*Fed. Proc.* **16,** *259* (*1957*)) have also demonstrated the conversion of xanthurenic acid to 8-hydroxyquinaldic acid by dehydroxylation.

While it is too early to fully appraise the significance of these recently discovered biochemical reactions in metabolism, at least one potentially practical use of the new knowledge has been suggested by M. D. Armstrong and A. McMillan (*Ibid.*, **16,** *146* (*1957*)). They found that 3,4-dihydroxymandelic acid, a metabolite of norepinephrine, is methylated to form the stable 3-methoxy-4-hydroxymandelic acid which is excreted in the urine and accounts for 30% of an administered dose of norepinephrine. Since the amount of 3-methoxy-4-hydroxymandelic acid excreted by two patients with pheochromocytomas was reduced substantially postoperatively, they have proposed determination of this compound in lieu of catechol amines for the detection of these tumors.

THE METABOLIC ROLE OF VITAMIN B_{12}

Although vitamin B_{12} has been isolated in pure form and its chemical structure determined, its exact biochemical role has not been eludicated. The vitamin has been implicated in two major metabolic areas, the biosynthesis of nucleic acids and methionine synthesis.

It was observed several years ago that in certain microorganisms vitamin B_{12} could be replaced by thymidine, suggesting that the vitamin was functioning in the biosynthesis of this nucleoside (*Nutrition Reviews* **7,** *19* (*1949*)). More recently, M. Downing and B. S. Schweigert (*J. Biol. Chem.* **229,** *521* (*1956*)) have investigated this phenomenon in detail. *Lactobacillus leichmannii* may be grown with B_{12} in the medium, or with any of several deoxyribosides in the absence of vitamin B_{12}. In order to determine if this observation actually reflected a role of vitamin B_{12} in the synthesis of the nucleosides, the organism was grown in the presence of C^{14}-labeled thymidine, with and without B_{12}. After maximum growth was obtained in each of the tubes the deoxyribonucleotides were isolated for C^{14} assay. The nucleotides isolated from the bacteria grown in the presence of vitamin B_{12} were of much lower specific activity than those isolated from bacteria grown without B_{12}. The authors considered that this reflected a greater dilution of the added C^{14} nucleoside as a result of greater nucleotide synthesis when B_{12} was present in the medium. Since all deoxy-

nucleosides will replace B_{12} for *L. leichmannii*, it was suggested that B_{12} functions in the synthesis or utilization of deoxyribose in the organism.

It has been reported that either thymine or thymidine administration to a pernicious anemia patient will result in a reticulocyte response (*Nutrition Reviews* **8,** *20* (*1950*)). This suggests a role of B_{12} in thymidine synthesis but since thymine is also effective does not point to a role of the vitamin in deoxyribose synthesis. Although vitamin B_{12} has been implicated in deoxyribonucleic acid metabolism it remains to be proved that the vitamin has any specific effect on the synthesis of this important tissue constituent.

The role of vitamin B_{12} in methionine metabolism has been extensively investigated. The suggested effects fall into 3 categories: transmethylation, the reduction of sulfhydryl compounds, and methyl synthesis.

Although there is evidence from both *in vivo* and *in vitro* experiments that B_{12} is concerned in transmethylation (*Nutrition Reviews* **8,** *301* (*1950*)) many negative results have been reported. In general it is considered that vitamin B_{12} is not concerned in transmethylation per se.

The effects of vitamin B_{12} on the reduction of disulfide groups have been reviewed (*Nutrition Reviews* **11,** *304* (*1953*)). There is considerable evidence that in B_{12} deficiency the reduction of disulfide groups to sulfhydryl groups is impaired. Since methionine formation requires the sulfhydryl group of homocysteine, an effect of B_{12} on maintaining this reduced form could explain the effects of the vitamin on methionine metabolism.

The relationship of vitamin B_{12} to methyl synthesis has been studied in animal and microbial experiments. A considerable body of evidence from Arnstein's laboratory has implicated B_{12} in methyl synthesis from formate, glycine or serine by rats (*Nutrition Reviews* **12,** *218* (*1954*)). In contrast, no evidence was obtained to implicate B_{12} in the conversion of glycine to serine (H. R. V. Arnstein and V. Stankovic, *Biochem. J.* **62,** *190* (*1956*)).

Perhaps the best evidence linking B_{12} to methyl synthesis has come from experiments using microorganisms (C. W. Helleiner and D. D. Woods, *Biochem. J.* **63,** *26* (*1956*)). Cell free extracts of an *E. coli* mutant requiring either methionine or B_{12} for growth were incubated with homocysteine, serine, $MgCl_2$, ATP and a heated extract of *E. coli*. Under such conditions the addition of catalytic quantities of B_{12} increased the rate of methionine synthesis four-fold. This observation suggests that B_{12} is necessary for the synthesis of methyl groups from serine.

In a recent report R. J. Young *et al.* (*J. Biol. Chem.* **224,** *341* (*1957*)) studied the effects of vitamin B_{12} on methyl synthesis in intact rats. Weanling rats were given a basal diet deficient in choline, methionine, and B_{12}. The effects of B_{12} on growth and liver fat were studied in the presence of supplements of betaine, monomethylaminoethanol, and choline. Growth rate was considered to reflect methionine synthesis, and reduction in liver fat was considered to reflect choline synthesis.

The effect of B_{12} on liver fat is difficult to interpret because the B_{12} supplemented animals usually grew faster. Fatty livers are more apt to occur in rapidly growing animals. The growth data in general were compatible with a role of vitamin B_{12} in methyl synthesis. The growth response to B_{12} was greatest in the methyl deficient animals and least in animals receiving supplements of betaine or choline.

From a consideration of all available evidence it appears likely that vitamin B_{12} is required for methyl synthesis. It should be pointed out, however, that such an effect could be the result of a role of the vitamin in maintaining sulfhydryl groups in the reduced state. Final proof of the biochemical function of vitamin B_{12} must come from experiments with purified enzyme systems.

GLUTAMATE AND ARGININE TREATMENT OF AMMONIUM TOXICITY

There is mounting evidence that some of the neurologic manifestations of severe liver disease, such as flapping tremor, drowsiness, confusion and disorientation, may be the result of an elevation of the blood ammonium concentration. A similar condition occurs in dogs in which the portal vein has been anastomosed with the inferior vena cava (Eck fistula) when fed high protein diets. The finding of high blood ammonium concentrations in these animals, as well as in certain patients with hepatic coma, has led to the view that the neurologic findings are a toxic result of the ammonium ion. The possible hazard of high protein diets for certain patients with chronic liver disease has been reviewed previously (*Nutrition Reviews* **14,** *74 (1956)*).

The recognition of the possible danger of ammonium toxicity has led to therapeutic efforts to control this biochemical abnormality. The possible benefit of glutamate in controlling ammonium accumulation in experimental animals was reported by M. R. Sapirstein (*Proc. Soc. Exp. Biol. Med.* **52,** *334 (1943)*). J. M. Walsche (*Lancet* **I,** *1075 (1953)*) reported on the use of this amino acid in cases of hepatic coma. It was argued that brain slices have the ability to form glutamine from glutamic acid and ammonia. The provision of exogenous glutamate would therefore promote the utilization of ammonia in the brain during hepatic coma. Promising results were observed in a small group of patients.

The clinical results of other workers, however, has been conflicting. A partial explanation of the contradictory findings has been provided by W. V. McDermott, Jr. and his co-workers (*New Engl. J. Med.* **253,** *1093 (1955)*). Little benefit was observed in patients with advanced liver disease who had central nervous system signs, but without a clear-cut predisposing stress such as excessive protein absorption from the gut or a history of treatment with either ammonium chloride or Diamox. When glutamate was given to patients who had had such an exogenous supply of potential ammonium producing material, there were demonstrable lowerings of the blood ammonium concentration and, in many cases, clinical improvement. Benefit was also seen in patients with chronic encephalopathy who had mild to moderate symptoms with relatively static cirrhosis. The use of sodium glutamate has been disappointing to the clinician since the patients most in need of help are helped little. The neurologic symptoms of patients with exogenous etiologic factors can usually be managed by other means.

Investigative interest is now being shown in the use of arginine to counteract ammonium toxicity. The rationale for this use is based on the conversion of arginine to hepatic ornithine. This latter amino acid partakes in the Krebs urea cycle, whereby carbon dioxide and ammonia are converted to urea. In the experiments of J. S. Najarian and H. A. Harper (*Proc. Soc. Exp. Biol. Med.* **92,** *560 (1956)*), arginine proved superior to glutamate in controlling the blood ammonia level in dogs receiving ammonium chloride. Comparable results were obtained by J. Greenstein *et al.* (*Arch. Biochem. Biophys.* **64,** *342 (1956)*) in rats receiving LD_{50} and $LD_{99.9}$ amounts of ammonium chloride. Although l-citrulline and l-ornithine had some effect, the greatest protection was observed when l-arginine was given prior to the ammonium chloride. Liver slices from the rats receiving l-arginine had an enhanced ability to convert ammonia into urea *in vitro*. These same investigators (J. P. Ruisseau *et al.*, *Ibid.* **64,** *355 (1956)*) demonstrated that the protective effect of l-arginine was associated with a lowering of the blood ammonium concentration. The toxicity of large doses of other essential amino acids could be related to elevations in the blood ammonium ion and was likewise aborted by prior administration of l-arginine.

The effect of arginine infusions on the

blood ammonium and neurologic manifestations of 15 patients with encephalopathy secondary to severe liver disease has been described by J. S. Najarian and H. A. Harper (*Am. J. Med.* **21,** *832 (1956)*). Twenty-five grams of l-arginine were given intravenously for each treatment. Sharp drops in blood ammonium levels were observed in most patients after the infusion. A rise in blood urea was observed often, and was cited as evidence that the benefit of arginine had been exerted by way of the Krebs urea cycle. Such experiments are difficult to interpret because the patients are often acutely ill and show a number of pathological entities. No evidence was provided to support the superiority of arginine over glutamate.

Such a direct comparison has been carried out in man by S. P. Bessman, S. Shear, and J. Fitzgerald (*New Engl. J. Med.* **256,** *941 (1957)*). Controlled studies were carried out in two patients with cirrhosis of the liver and in two patients without liver disease. Ammonium chloride was given intravenously alone, or with either sodium glutamate or arginine hydrochloride. Each patient received all three treatments. Arginine and glutamate did not promote the disappearance of ammonia from the arterial blood of the two control subjects. In the cirrhotic patients arginine had a slight effect and glutamate had a pronounced effect in lowering the arterial ammonium concentration. The authors suggest that cirrhotic patients have adapted enzymatically to the formation of glutamine, which substitutes for normal urea synthesis.

The ultimate usefulness of amino acid therapy of hepatic encephalopathy cannot be predicted on the basis of today's evidence. Under certain conditions glutamate and arginine can lower the blood ammonium level of man and experimental animals. The choice between the two amino acids, the proper dose and the duration of treatment can not be considered established. When gross disorganization of hepatic function exists, clinical benefit may be meager. This may be particularly true in the case of arginine whose therapeutic effect is dependent on an hepatic enzyme system.

THE INTESTINAL ABSORPTION OF GLUCOSE

The way in which glucose is absorbed from the intestine has been a subject of experimental interest ever since E. Bárány and E. Sperber demonstrated that it disappeared from the gut even though the blood concentration was higher (*Skand. Arch. Physiol.* **81,** *290 (1939)*). Although "active absorption" may have been an adequate statement some years ago, the known complexities of carbohydrate metabolism make one realize that this must be a great oversimplification.

R. M. Atkinson, B. J. Parsons, and D. H. Smyth (*J. Physiol.* **135,** *581 (1957)*) point out that "it has not even been definitely established in what form the actively transferred glucose enters the mesenteric blood". They have investigated the matter by studying the fate of glucose (uniformly labeled with C^{14}) absorbed from a loop of dog's intestine, finding that 65 to 80 per cent of it was accounted for in mesenteric venous blood as glucose and 7 to 17 per cent as lactic acid.

Dogs were anesthetized with intravenous pentobarbitone sodium, the abdomen was opened, and a 20 cm. loop of intestine was selected. The animal was heparinized, the vein draining the loop was cannulated, and the loop was washed out with warm saline and tied off from the rest of the intestine. Maintenance of normal blood pressure and blood flow obviated the development of anoxia. The venous cannula was attached to a polyethylene tube brought out through a hole in the abdominal wall. Immediately outside it formed a coil cooled by ice water. After passing through a dropping-chamber, the blood was collected under oil in an iced

cylinder to inhibit glycolysis; fluoride was used in addition in some experiments. When blood collection was established the test solution of glucose was injected into the intestinal loop, which was replaced in the abdomen. Venous blood was collected for 15 to 60 minutes, after which the contents of the loop were washed out and collected. "True glucose" was determined by the method of Shaffer and Somogyi, coupled with a microfermentation method. Other substances were measured by appropriate methods.

Various samples were so treated that the C^{14} was finally incorporated into barium carbonate or into a 2,4-dinitrophenylhydrazone, which was plated and counted. Relative specific activities were then calculated. In particular, glucose was determined by anaerobic yeast fermentation and precipitation of the evolved carbon dioxide as barium carbonate. In a second method the glucose was finally burned to form carbon dioxide, which was converted to barium carbonate.

The authors report eight experiments of 30 to 60 minutes' duration. The initial concentration of glucose in the intestine was 173 to 390 mg. per cent, and absorption proceeded almost to completion (less than 1 mg. usually remaining in 1 to 3 ml. of fluid). It is inferred that absorption must have occurred against a concentration gradient. In one experiment the blood glucose was artificially raised above that in the intestine and the authors conclude that absorption against the gradient took place throughout the experiment.

The absorbed C^{14} was recovered as glucose to the extent of 65 to 80 per cent and as lactic acid (7 to 17 per cent) in the eight experiments. The percentages for alanine (2, 3 per cent), pyruvic acid (1, 2 per cent) and carbon dioxide (3, 3 per cent) were determined in only two cases. Total recovery of absorbed C^{14} ranged from 72 to 93 per cent in the eight experiments. Negligible amounts of labeled lactic acid, pyruvic acid, and carbon dioxide remained in the intestinal lumen. Extracts of the intestinal wall similarly contained unimportant amounts of labeled glucose, lactic acid and alanine. Since the specific activity of the glucose in the intestine at the end of the experiment was almost the same as at the beginning, the evidence indicated that reverse transport of glucose from blood to intestinal lumen did not occur.

The authors have, it appears, refuted rather nicely the idea that most glucose is normally absorbed as lactic acid. T. H. Wilson and G. Wiseman (*Ibid.* **123,** *116 (1954)*) came to this conclusion studying the removed intestine which had been turned inside out. H. Newey, D. H. Smyth (one of the present authors) and B. C. Whaler (*Ibid.* **129,** *1 (1955)*) had similar results *in vitro*. S. Hestrin-Lerner and B. Shapiro (*Biochim. et biophys. acta* **13,** *54 (1954)*) studied the rat intestine *in vivo* and *in vitro*, and concluded that most glucose was absorbed into mesenteric blood in an unidentified form. Their measurements of lactic acid do not appear very complete, and they place heavy emphasis on failure to demonstrate an increase in mesenteric blood glucose, calculating a considerable "expected" rise based on glucose absorption and an assumed blood flow.

A number of experiments were not included in Atkinson, Parsons, and Smyth's tables because of inadequate blood flow or a congested appearance of the mucosa. Higher percentages of the absorbed glucose C^{14} (30 to 40 per cent) were recovered as lactic acid in these instances than in the satisfactory experiments. One concludes that the more anoxemia there is the more lactate is found, and one may speculate further as to whether the 7 to 17 per cent values in the satisfactory experiments may not be due to slightly unphysiologic conditions (*i.e.* tissue anoxia) associated with the procedure. Perhaps the intact animal would have even smaller amounts of glucose absorbed as lactate or possibly it would have none.

The experiments of Atkinson, Parsons and Smyth have been well planned to overcome a

number of difficulties besetting previous workers who sought to learn how glucose is absorbed. Since arterial blood contains abundant glucose, high concentrations in the intestine have been necessary to show a significant increase in mesenteric venous glucose. Enormous concentration gradients have obtained in such experiments, favoring diffusion. As the authors point out, such experiments could not answer the question of active versus passive transport of glucose. The isotope technique seems ideally suited to overcoming these difficulties and to determining how much of the absorbed glucose is accountable, and in what form. It is to be hoped that more papers from these authors may be forthcoming on this subject.

Although Atkinson, Parsons and Smyth have accounted for "up to 90 per cent of the glucose disappearing from the intestinal lumen . . . in the mesenteric venous blood as either glucose or lactic acid", it is no criticism of their work to point out that the locus of the problem has been placed within the cell. A host of cytochemical transformations is still possible between the limiting facts that glucose is presented to the intestinal surface of the cell and glucose emerges from the opposite side into the blood. Indeed, from our present knowledge of the variety of transformations and combinations to which this substance is liable, we can almost predict that glucose crosses the cell membrane and gets across the cell in some combined form. Whether the carbon chain is split and then recombined or some combination with phosphate or a coenzyme occurs, we cannot say. The use of uniformly labeled glucose, as in the work here reviewed, would not test the first possibility, as all carbon atoms of the chain are labeled in the same proportion. But glucose labeled, say, in the C_1 position, if split into triose and then recombined, would be expected to yield glucose labeled in the C_1 and C_4 positions; if not, it should yield only glucose labeled in the C_1 position.

Interesting studies on this point and on the nature of the glucose transport system of renal tubular epithelium have been reported by F. P. Chinard, W. R. Taylor, M. F. Nolan, and T. Enns (*Science* **125,** *736 (1957)*). They found that when glucose labeled with C^{14} in the C_1 position was injected into the renal artery of the anesthetized dog, essentially all the radioactivity in the renal vein blood (containing glucose returned through the tubular epithelium) resided in the C_1 position. Given the calculated glucose transit time across the proximal tubular epithelium, the thickness of the cell, and assuming that a glucose complex traverses the cell by diffusion, these authors calculated a diffusion coefficient. Next, assuming the viscosity of cytoplasm to be ten times that of water, they calculated the particle weight of the hypothetical complex, arriving at a figure of the order of 2000 g. per mole. The authors suggest, therefore, that "the glucose-carrier complex, if such exists, is not a macromolecule and that the transport does not directly involve organized intracellular particles, such as mitochondria". On the basis of other experiments, Chinard, Taylor, Nolan and Enns tentatively conclude that "the transport system is of the membrane carrier rather than of the cytoplasm carrier variety".

It will be interesting to follow future work on glucose absorption and transport and learn to what extent intestinal and renal tubular epithelium are alike in this function. With the great advances being made by the histochemists in identifying and localizing such fleeting and soluble substances as the carbohydrates and hexose-6 phosphatase, it may even be possible some day to follow the glucose as it gets across the cell, showing that it localizes as this or that compound, accumulating to form a diffusion gradient toward the blood, or that it does not.

In summary, the absorption of C^{14}-labeled glucose from intestinal loops of the dog has been studied and up to 93 per cent of the absorbed material accounted for (65 to 80 per cent as glucose and 7 to 17 per cent as lactic acid) in the mesenteric venous blood which it has reached, often, against a

diffusion gradient. Studies on renal tubular epithelium are reviewed indicating that in this cell, at least, the glucose molecule is reabsorbed without splitting, and that the intracellular carrier complex is probably not a macromolecule or larger organized particle.

HYDROCORTISONE ADMINISTRATION DURING PREGNANCY: ITS RELATION TO THE OCCURRENCE OF CLEFT PLATE

Studies carried out on inbred strains of mice have demonstrated that cortisone administration for several days during gestation resulted in a high incidence of cleft palate in the progeny (*Nutrition Reviews* **14,** *115* (*1956*)) when administered during a period after the eleventh day of gestation.

A situation similar to that observed in mice can be considered in the human in relation to two isolated cases of cleft palate in the newborn reported in association with cortisone therapy given to the mother during the first two months of pregnancy (*Lancet* **I,** *1045* (*1956*); **II,** *730* (*1956*)).

T. H. Ingalls, *et al.*, demonstrated that hypoxia inflicted in mice at different periods during gestation also resulted in the production of cleft palate in the progeny. The critical time for the stress to be applied with the highest incidence of this congenital defect was found to be around the fifteenth day of gestation.

In another study, T. H. Ingalls and F. J. Curley (*New Eng. J. Med.* **256,** *1035* (*1957*)) took white mice of unpedigreed ancestry in order to approximate more closely the mixed human hereditary factors. In contrast to the program of cortisone administration over several days, as carried out during previous studies, they administered hydrocortisone on a single day between the ninth and seventeenth days of gestation. The incidence of spontaneous cleft palate in this lot of mice had been found to be less than 0.2 per cent. The incidence of induced cleft palate occurred when hydrocortisone was administered between the tenth and sixteenth days. The highest incidence occurred when it was administered on the twelfth and thirteenth days: 47 per cent and 52 per cent respectively, following a dose of 5 mg., 100 per cent and 64 per cent respectively, following a dose of 10 mg. The incidence on the other days was much less and no mouse manifested a defect from treatment when it was administered before the tenth, and on or after the sixteenth, day of gestation. This indicates that a significant change in risk can be introduced by a difference of as little as one day in the administration of hydrocortisone.

A discussion of the type of anatomical lesion follows, and reveals a difference between the inherited and the hydrocortisone-induced cleft palate noted in a preliminary study conducted on A/Jax mice. It was found that the inherited defect is invariably associated with harelip and alveolar involvement, whereas that induced by hydrocortisone or hypoxia was always midline, without involvement of the lip or alveolus. Extrapolation is made to the human population where it is mentioned that in a majority of the cases anterolateral cleft palate with harelip is inherited, whereas posterior median cleavages occur as isolated events. The two reported human instances mentioned above are of the latter type.

NOTES

Letter to the Editor

Dear Sir:

I was interested in your review on X disease in *Nutrition Reviews* **11,** *97 (1953).* The explanation you give of the etiology of this disease is particularly interesting to me, as you refer to a work I did in collaboration with my friend Professor Paul Meunier, who died in February, 1954. You suggest that X disease is caused by an antivitamin A. I think another mechanism is perhaps possible.

Chloro naphthalene is detoxicated in three ways:

1. by combination with sulfur amino acids (Methionine, or cystine), to form naphthylmercapturic acid.
2. by sulfoconjugation, to form naphthyl ethereal sulphate.
3. by glucuronoconjugation, to form naphthylglucuronide.

Meunier and I have shown that with the rat, Bromobenzene detoxication follows in the same way and causes a bigger consumption of vitamin A for animal organisms.

How could this be explained? Perhaps vitamin A intervenes in synthesis from which result new and less toxic substances (*i.e.* mercapturic acid, sulfoconjugates, glucuronoconjugates). At the same time, perhaps vitamin A turns detoxication towards sulfoconjugation or glucuroconjugation if sulfur amino acids from the ration are not sufficient.

In the case of Chloro naphthalene we may suppose that this product can first disturb certain syntheses made by rumen bacteria. Then methionine synthetized is not sufficient for a good synthesis of mercapturic acid and glucuronoconjugation is necessary, but very quickly available glucuronic acid is spent. The vitamin A can very well be used in glucuronic acid formation which perhaps ruminants cannot dispose of in great quantities since their particular physiology implies a slow degradation of polysaccharides which in bovines implies a rather low rate of glycemia and, on the other hand, a large amount of acetic acid in the blood. All this might lead to greater consumption by stock of vitamin A in bovines intoxicated by Chloro naphthalene; hence, the symptoms noted.

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Seine, France

Selenium in the Prevention of Liver Necrosis

It was shown some years ago that when rats were fed a diet containing large quantities of torula yeast, liver necrosis developed. This condition could be prevented by either vitamin E or cystine, or by a substance present in other yeasts and designated factor 3 (*Nutrition Reviews* **10,** *288 (1952)*). The chemical nature of factor 3 has been the subject of considerable research in the six years since its discovery and appears to have been elucidated finally by K. Schwarz and C. M. Foltz (*J. Am. Chem. Soc.*, **79,** *3292 (1957)*).

In the process of fractionating factor 3 concentrates it was noted that the addition of alkali to active fractions resulted in the production of a garlic-like odor. This observation suggested the presence of selenium, and various factor 3 preparations were analyzed for this element. It was found that the selenium concentration quite closely paralleled the biological activity of the various fractions. The necrosis-preventing action of selenium was then tested. Rats were fed the torula yeast diet which resulted in 100 per cent incidence of necrosis. The addition of 0.0133 mg. per cent sodium selenite to this diet resulted in complete protection. Potassium selenate was also effective although larger quantities were required.

From these results it appears that the

activity of factor 3 preparations is due to the organically bound selenium, and that selenium is an essential trace element.

The Conduct of Clinical Trials

At the request of the Committee on Nutrition of the American Academy of Pediatrics, C. U. Lowe and V. Pessin have prepared a report entitled "The Conduct of Clinical Trials of Substances Proposed for the Nutrition of Infants and Children: Including a Critique of the Nitrogen Balance Technique for Measuring Protein Anabolism" (*Pediatrics* **19,** *693 (1957)*). This report can be highly recommended to all those dealing with the assessment of nutrients in children.

The report is divided into sections on population, controls, criteria of response, sample size and duration of study, and the form of reporting results. Under criteria for judging response is a rather detailed and critical comment on the value of the nitrogen balance technique. These authors emphasize the necessity for direct analysis of diets as well as excreta, the publication of data concerning the precision of the laboratory procedures, and the measurement of at least one substance other than nitrogen from which a theoretical nitrogen retention could be calculated. Consideration is also given to the design of such clinical trials, particularly determination of the size of the sample necessary for the statistical demonstration of differences of varying magnitudes.

While this report was designed primarily for those undertaking nutritional investigations in children, it will also be of great help in the interpretation and evaluation of such reports.

Recent Nutrition Books

Medicine in a Changing Society. Number XVIII of The New York Academy of Medicine Lectures to the Laity. Iago Galdston, M.D., Editor. International Universities Press, Inc., New York, 1956. Pp. 161. Price $3.00.

Human Protein Requirements and Their Fulfilment in Practice. Proceedings of a Conference in Princeton, United States (1955). Sponsored jointly by The Food and Agriculture Organization of the U. N. World Health Organization and Josiah Macy Jr. Foundation, New York. The FAO Sales Agent in the USA is the Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Pp. 176. Price $2.00.

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